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Formation of *o*-Nitrosobenzaldehyde from Hydrolysis of *o*-Nitrobenzyl Tosylate. Evidence of Intramolecular Nucleophilic Interaction.

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Abstract: Hydrolysis of *o*-nitrobenzyl tosylate in CH_3CN:H_2O (1:1, v/v) gave *o*-nitrobenzyl alcohol and *o*-nitrosobenzaldehyde in 1.8 : 1 ratio. Formation of *o*-nitrosobenzaldehyde indicates that the nitro group participates in the leaving of the tosylate group. *o*-Nitrosobenzaldehyde was reduced by biological thiols to give *o*-aminobenzaldehyde. Reaction of *o*-nitrosobenzaldehyde with 1 mol of benzylamine afforded 3-(N-benzylamino)anthranil (or its tautomer) as a major product. Published by Elsevier Science Ltd.

o-, *m*-, and *p*-Nitrotoluenes are a group of industrially important chemicals used in the production of dyes, rubber, and agricultural chemicals with total annual production in the United States estimated to be 44 million pounds.[1] Toxicology studies of these chemicals in F344 rats have clearly shown that *o*-nitrotoluene is the most toxic isomer. It produces more damage to the liver and kidney than *m*- and *p*-isomers and is the only isomer that causes mesotheliomas in male rats.[2] All three nitrotoluenes are initially metabolized to the corresponding nitrobenzyl alcohols followed either by further oxidation, or conjugation with small polar endogenous entities such as glucuronic acid or sulfate.[3] An unexplored pathway possibly responsible for the toxicity of *o*-nitrotoluene is an intramolecular reaction between the *o*-nitro group and the benzylic carbon bearing a leaving group such as sulfate to give a reactive metabolite. There is literature precedent for formation of reactive products from intramolecular reaction between a benzylic carbon substituted with a leaving group and an *o*-nitro group.[4,5] For example, in the reaction of *o*-nitrobenzyl alcohol with strong acids,[4,5] displacement of H_2O in the protonated alcohol by the nitro group gave the C-protonated conjugate acid of anthranil N-oxide (**1**$^+$) (Scheme 1).[4] Loss of H^- from **1**$^+$ would give anthranil N-oxide (**2a**) or its open form, *o*-nitrosobenzaldehyde (**2**). However, **2** was not detected under the highly acidic conditions.[4,5] We wish to report isolation of *o*-nitrosobenzaldehyde (**2**) under neutral reaction conditions when using o-nitrobenzyl tosylate as a model compound for our hydrolysis study.

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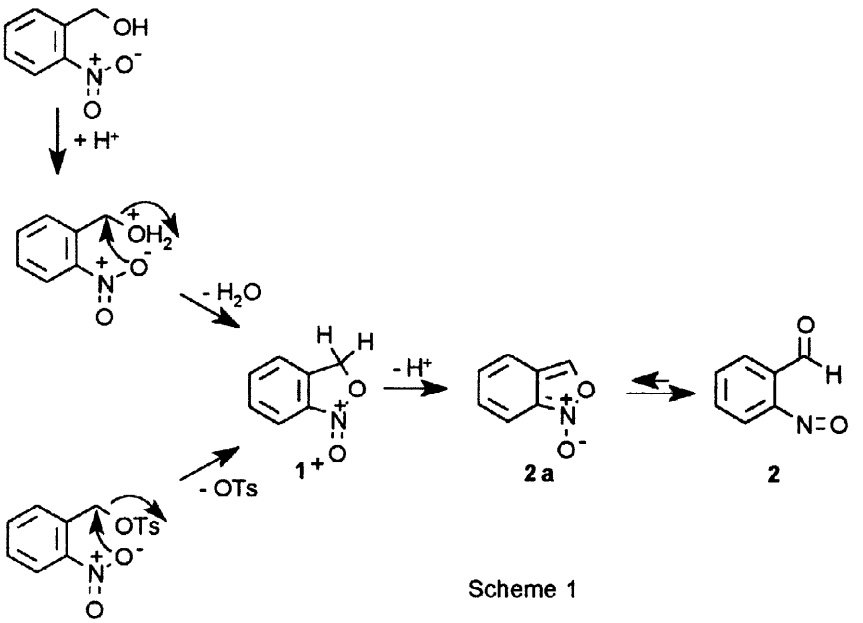
Hydrolysis of *p*-nitrobenzyl tosylate was also studied in order to compare the isomeric difference between *o*- and *p*-isomers.



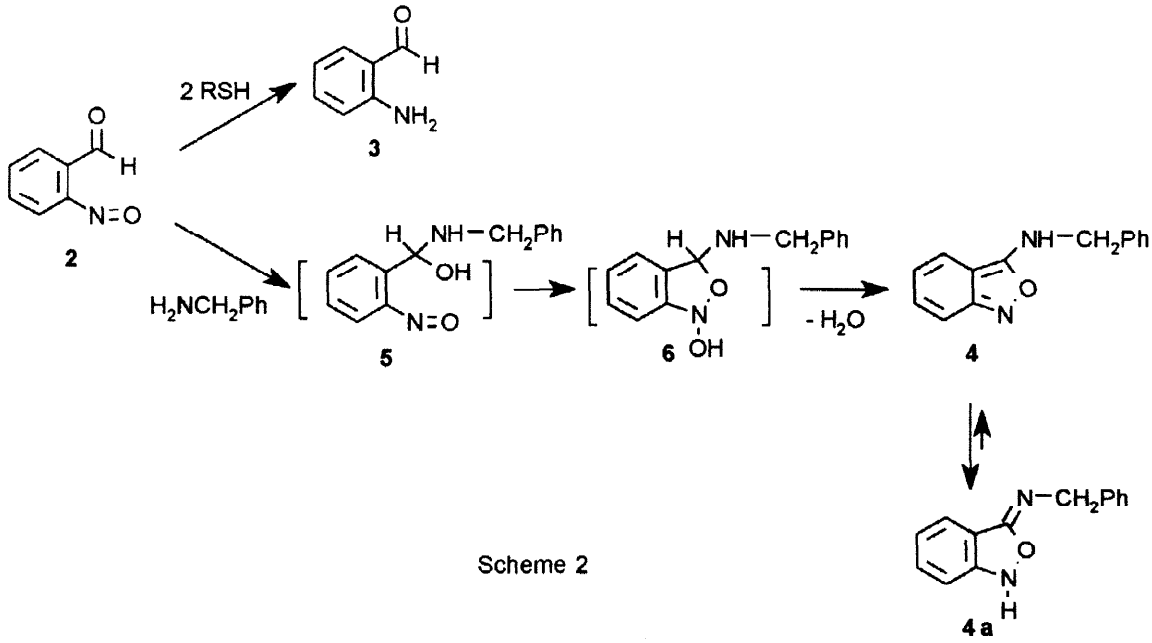
Scheme 1

o- and *p*-Nitrobenzyl tosylates were prepared[6] and were hydrolyzed in CH_3CN-H_2O (1:1, v/v) at 37°C and 47.5°C. The progress of the reaction was followed by HPLC analysis with UV detection at 254 nm. *p*-Toluenesulfonic acid and *p*-nitrobenzyl alcohol were obtained from *p*-nitrobenzyl tosylate as expected. However, hydrolysis of *o*-nitrobenzyl tosylate gave not only *p*-toluenesulfonic acid and *o*-nitrobenzyl alcohol, but also a third product. This product was isolated by HPLC; its ^1H NMR spectrum was consistent with that of *o*-nitrosobenzaldehyde (**2**) (Scheme 1).[4,7] *o*-Nitrosobenzaldehyde isolated from hydrolysis of *o*-nitrobenzyl tosylate was spectrally and chromatographically identical to the product obtained from an independent synthesis.[7,8] Formation of *o*-nitrobenzyl alcohol could have arisen from the addition of H_2O to $\mathbf{1}^+$ or direct nucleophilic displacement of the tosylate group in *o*-nitrobenzyl tosylate with H_2O, but we have not been able to differentiate between these pathways.

The product ratio and rates of hydrolysis of *o*- and *p*-nitrobenzyl tosylates were measured by HPLC. The product ratio from hydrolysis of *o*-nitrobenzyl tosylate in CH_3CN-H_2O (1:1) after 6 hours at 47.5°C was determined to be *o*-nitrobenzyl alcohol : *o*-nitrosobenzaldehyde : *o*-nitrobenzyl tosylate = 35 % : 20 % : 45 %. After 6 hours at 37°C, the ratio is 15 % : 8 % : 71 %. Under these hydrolysis conditions, decomposition of **2** began to be detected after 6 hours, although it was stable in the solid form. A rate constant of $(5.83 \pm 0.06) \times 10^{-5}$ s^{-1} (n = 3) was measured for the disappearance of *p*-nitrobenzyl tosylate in CH_3CN-H_2O (1:1) at 47.5°C, which is in agreement with a value of 4.60×10^{-5} s^{-1} at 45°C reported for the same reaction.[9] The corresponding rate constant for the disappearance of *o*-nitrobenzyl tosylate at 47.5°C was $(3.63 \pm 0.04) \times 10^{-5}$ s^{-1} (n = 3); slower than the *p*-isomer by a factor of 0.62. Reported ratios of rate constants (*o*-isomer/*p*-isomer) are 0.88 for disappearance

of both nitrobenzyl chlorides in acetone-H_2O (1:1, v/v) at 60°C[10] and nitrobenzyl tosylates in acetone-H_2O (9:1, v/v) at 32.5°C.[6] Based on this rate information, it seems unlikely that the difference in toxicity is due to a greater reactivity of *o- vs. p*-substituted nitrobenzyl conjugates.

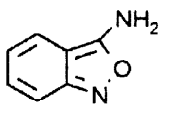
Reactivity of *o*-nitrosobenzaldehyde toward nucleophiles containing sulfhydryl or amino groups was investigated. *o*-Nitrosobenzaldehyde reacts rapidly with biological thiols, including glutathione, cysteine, and N-acetylcysteine in CH_3CN-H_2O (1:1, v/v) at room temperature to give the same product upon HPLC analysis. This product was identified as *o*-aminobenzaldehyde (**3**) by comparison of spectroscopic and chromatographic data with an authentic sample (Scheme 2). The mechanism for formation of *o*-aminobenzaldehyde is probably similar to that proposed by Ellis *et al.*[11] for the formation of nitroanilines from reduction of nitrosonitrobenzenes with glutathione. Both *o*-nitroaniline and *o*-nitrophenylhydroxylamine were isolated from the reaction of *o*-nitrosonitrobenzene with glutathione.[11] The intermediate, glutathion-S-yl conjugate, was also observed in solution. In our reactions, neither *o*-hydroxylaminobenzaldehyde nor the glutathion-S-yl conjugate was detected by HPLC analysis.



Scheme 2

The reaction of *o*-nitrosobenzaldehyde with 1 mol of benzylamine was carried out in $CHCl_3$ at room temperature. TLC (diethyl ether-$CHCl_3$ (1:9)) of the reaction mixture showed that *o*-nitrosobenzaldehyde (R_f = 0.68) disappeared after a few minutes and a major product with R_f = 0.09 was formed. The isolated product was identified as either 3-(N-benzylamino)anthranil (**4**) or its tautomer **4a** on the basis of MS and NMR spectra (Scheme 2). Positive ion ESI-MS analysis of this material gave a molecular ion peak at m/z 225. The peak at m/z 225 is consistent with loss of one H_2O from a 1:1 adduct of *o*-nitrosobenzaldehyde and benzylamine. ^1H NMR (360 MHz, $CDCl_3$) spectrum of this product showed signals at δ 7.88 (d, J = 7.91 Hz, 1H), 7.48 (t, J = 7.77 Hz, 1H), 7.36-7.33 (m, 5H, benzylic-Ph), 7.21 (t, J = 7.61 Hz, 1H), 7.13 (d, J = 8.21 Hz, 1H), 6.73 (br. s, 1H,

NH), 5.03 (s, 2H, benzylic-CH_2). The peak at 6.73 ppm exchanged with D_2O, a strong indication of an exchangeable secondary amino proton as part of the structure. The doublet at δ 6.5 for the proton *ortho* to the nitroso group and the singlet at around δ 12.0 for the aldehyde proton in *o*-nitrosobenzaldehyde diappeared, which is consistent with the absence of both nitroso and aldehyde functionality in the product. Formation of **4** (and **4a**) can be rationalized by addition of the amine to the aldehyde carbonyl group to give a hemiaminal (**5**), followed by intramolecular addition of the resulting hydroxyl group to the nitroso group to give a cyclic intermediate **6**. Transannular dehydration of **6** affords **4**, which could rearrange to its tautomer **4a** (Scheme 2). Our product has the same MS and ^1H NMR spectra as the material obtained from the cleavage of 1-(N-benzylamino)-2'-nitrobenzylphosphonate in a basic medium.[12] Boduszek *et al.* assigned the structure of the cleavage product to be **4** by comparison with unsubstituted 3-aminoanthranil, whose structure has been assigned to be 7, and not **7a**, based on its IR spectrum following partial H-D exchange.[13] **7** is bright yellow in EtOH and has a UV-maximum at 367 nm. By comparison, the colorless compound from our reaction has UV-maximum at 312 nm in EtOH (UV of **4a**: λ_{max} (ε) 217 nm (23000), 234 nm (14000), 312 nm (4400)). Therefore, we believe our product , and the product of Boduszek *et al.*, is more likely to be **4a** which lacks the anthranil chromophore.



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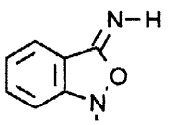

7a

We have shown that hydrolysis of *o*-nitrobenzyl tosylate afforded *o*-nitrosobenzaldehyde (**2**) as a result of intramolecular nucleophilic substitution. **2** was reduced quickly by biological thiols to give *o*-aminobenzaldehyde (**3**). A stable adduct **4a** was formed from the reaction of **2** with benzylamine. Studies are ongoing to explore biological significance of the above reactions.

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